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Guanylyl Cyclase: A Cell-Surface Receptor Throughout the Animal Kingdom

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Abstract. The enzyme guanylyl cyclase occurs in both soluble and membrane-associated forms, and catalyzes the formation of the second messenger cyclic GMP. The membrane forms of guanylyl cyclase encompass a family of cell-surface receptors that mediate the biological activity of a variety of peptide ligands. Representatives of this receptor family have been identified in such diverse species as rats and sea urchins. In echinoderm spermatozoa, guanylyl cyclase is a receptor for egg-associated peptides, eliciting species-specific effects, including changes in sperm respiration and motility, and a chemotactic response. In mammals, the family of guanylyl cyclases includes receptors for natriuretic peptides, as well as the target of bacterial heat-stable enterotoxins. The identification of natriuretic peptides in amphibians, birds, and fish suggests that guanylyl cyclase receptors play a role in osmotic balance in a variety of organisms. Molecular cloning evidence implies that homologs of the mammalian guanylyl cyclase receptors exist in other vertebrates, and perhaps even in invertebrates. Guanylyl cyclases thus represent an example of receptors that have diversified to serve both as external chemoreceptors and as internal hormone receptors.

Introduction

The production of cyclic GMP, an intracellular second messenger, is catalyzed by guanylyl cyclases. This enzyme family includes both soluble and particulate forms, and is widely distributed in various cell types throughout the animal kingdom. The soluble and particulate forms of the enzyme are distinctly different in both their structure and their mode of regulation. The soluble enzyme is a heterodimer composed of α and β subunits; tissue-specific isoforms of each subunit are known to exist (Schulz *et al.*, 1991). The activity of the soluble enzyme is regulated by diffusable substances, nitric oxide for example (Schulz *et al.*, 1991). The particulate enzyme, in contrast, is monomeric (although the active conformation may be multimeric), with a single transmembrane domain (Yuen and Garbers, 1992). A variety of studies have demonstrated that members of the particulate guanylyl cyclase family are receptors for such diverse ligands as echinoderm egg peptides, natriuretic peptides, and *Escherichia coli* heat-stable enterotoxin. Cyclic GMP generated in response to such stimuli is implicated in the regulation of ion channel function, and the modulation of cyclic nucleotide-dependent phosphodiesterase and protein kinase activity. This review will focus on the current understanding of the receptor guanylyl cyclases in mammals, and their counterparts in other species.

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Receptor Guanylyl Cyclases in Echinoderms

Particulate guanylyl cyclase was first characterized in, and purified from, the spermatozoa of sea urchins. This cell type is a rich source of the particulate form of the enzyme, and apparently contains no soluble enzyme. Studies of sea urchin spermatozoa were among the first to suggest that guanylyl cyclase might serve as a receptor for extracellular signals. Peptides were purified from sea urchin egg-conditioned medium that would elicit several biochemical and behavioral changes in spermatozoa of the homologous species. Exposure of spermatozoa to the peptides resact or speract (purified from *Arbacia punctulata* or *Strongylocentrotus purpuratus*, respectively, Table I) increases respiration and motility, elevates intracellular pH, alters ion fluxes, and elevates cyclic nucleotide levels (Garbers, 1989). In addition, resact is a potent chemoattractant for *Arbacia* spermatozoa (Ward *et al.*, 1985). Crosslinking studies demonstrated that resact apparently

Table I

Amino acid sequences of peptides known to activate particulate guanyly cyclases. Longer circulating forms of brain and C-type natriuretic peptide have been identified

I. Atrial Natriuretic Peptide	
Human/Porcine	S L R R S S C F G G R M D R I G A Q S G L G C N S F R Y
Rat	S L R R S S C F G G R I D R I G A Q S G L G C N S F R Y
Eel	S K S S S P C F G G K L D R I G S Y S G L G C N S R K
Bullfrog	S S D C F G S R I D R I G A Q S G M G C – G R R F
II. Brain Natriuretic Peptide	
Human	S P K M V Q G S G C F G R K M D R I S S S S G L G C K V L R R H
Porcine	S P K T M R D S G C F G R R L D R I G S L S G L G C N V L R R Y
Rat	N S K M A H S S S C F G Q K I D R I G A V S R L G C D G L R L F
Chicken	M M R D S G C F G R R I D R I G S L S G M G C N G S R K N
III. C-Type Natriuretic Peptide	
Porcine	G L S K G C F G L K L D R I G S M S G L G C
Killifish	G W N R G C F G L K L D R I G S M S G L G C
Eel	G W N R G C F G L K L D R I G S L S G L G C
IV. Heat-Stable Enterotoxins and Gastrointestinal Peptides	
Guanylin	P N T C E I C A Y A A C T G C
Escherichia coli ST_h	N S S N Y C C E L C C N P A C T G C Y
Escherichia coli ST_p	N T F Y C C E L C C N P A C A G C Y
Yersinia enterocolitica ST	V S S D W D C C D V C C N P A C A G C
Vibrio cholerae non-01 ST	I D C C E I C C N P A C F G C L N
V. Echinoderm Egg Peptides	
Speract (*Strongylocentrotus purpuratus*)	G F D L N G G G V G
Resact (*Arbacia punctulata*)	C V T G A P G C V G G G R L – NH_2
Mosact (*Clypeaster japonicus*)	D S D S A Q N L I G

binds to a 160,000 molecular weight protein that was shown to be guanylyl cyclase (Shimomura *et al.*, 1986). Speract, on the other hand, could be crosslinked to a 77,000 molecular weight protein (Dangott *et al.*, 1989). The relationship between the two crosslinked binding proteins is unclear, because both sea urchin species contain both proteins. The functional receptor may be composed of multiple subunits, only one of which is labeled by chemical crosslinking. The respective guanylyl cyclases and 77 kDa proteins have been cloned from both *Arbacia* and *Strongylocentrotus* (Singh *et al.*, 1988; Dangott *et al.*, 1989; Thorpe and Garbers, 1989; L. J. Dangott, pers. comm.). However, none of the clones conferred peptide binding or cyclase activity upon transfected mammalian cells. Incorrect processing of echinoderm transcripts may be responsible for the lack of functional expression in COS cells, or both proteins may be necessary for the expression of a peptide-binding complex.

Receptor Guanylyl Cyclases in Mammals

The cloning of sea urchin guanylyl cyclase provided a valuable probe for cloning mammalian forms of the enzyme. The amino acid sequence predicted by the sea urchin guanylyl cyclase cDNA contained a single putative transmembrane domain, suggesting a topography like that of the growth factor receptors: an extracellular ligand binding domain, and an intracellular regulatory/catalytic domain. The cDNA encoding the intracellular, presumably conserved, regulatory/catalytic domain was used as a probe to screen various cDNA libraries, and the first mammalian guanylyl cyclase (GC-A) was cloned from rat and human (Chinkers *et al.*, 1989; Lowe *et al.*, 1989). Reports in the literature had demonstrated the stimulation of guanylyl cyclase activity by atrial natriuretic peptide (ANP), and the copurification of ANP binding and guanylyl cyclase activities (reviewed in Schulz *et al.*, 1989a). Expression of cloned GC-A allowed direct testing of the hypothesis that guanylyl cyclase was an ANP receptor, and this was shown to be the case (Chinkers *et al.*, 1989; Lowe *et al.*, 1989). A number of natriuretic peptides have now been discovered, and the cloning of a second mammalian guanylyl cyclase (GC-B) has demonstrated the diversity within this receptor-enzyme family (Chang *et al.*, 1989; Schulz *et al.*, 1989b). GC-B also binds and is activated by natriuretic peptides. But although GC-A preferentially binds and responds to ANP, GC-B preferentially responds to C-type natriuretic peptide (CNP) (Koller *et al.*, 1991). Whether additional natriuretic peptide receptors exist is not yet known, but the existence of brain natriuretic peptide (BNP) suggests that at least one additional member of the family remains to be identified.

Heat-stable enterotoxins (ST) produced by several strains of pathogenic bacteria have long been known to

Table II

Alignment of partial-length guanylyl cyclase-receptor sequences with the homologous regions of the mammalian natriuretic and heat-stable enterotoxin receptors

Speract Receptor	AYMLVSGLPLRNGDRHAGQIASTAHHLLESVKGFIVPHKPRVFLKLRIGIHSGSCVAGVVGLT
Natriuretic Peptide and Related Receptors	
Rat GC-A	AYMVVSGLPVRNGQLHAREVARMALALLDAVRSFRIRHRPQEQLRLRIGIHTGPVCAGVVGLK
Rat GC-B	AYMVVSGLPGRNGQRHAPEIARMALALLDAVSSFRIRHRPHDQLRLRIGVHTGPVCAGVVGLK
Fish	AYMVVSGLPVRNGKLHAREIAGMSLALLEQVKTFKIRLRPNDQLRLRIGIHTGP
Xenopus	AYMVVSGLPVRNGKLHAREIARMSLALLDAVKSFKIRHRPDQQLGLRIGI
Sea urchin	AYMCVSGLPIRNGDFHAREIARMSLALLHEITSFRIRHRPEERLRLRIGVHTG
Earthworm	AYMVVSGLPVRNGKLHAREIARMSLALLDAVRSFKIRHRPNQQLRLRIGIHTGPVCAGVVGLK
Heat-Stable Enterotoxin and Related Receptors	
Rat GC-C	AYVVASGLPMRNGNRHAVDISKMALDILSFMGTFELEHLPGLPVWIRIGVHSGPCAAGVVGIK
Fish	AYMVASGLPKRNGDRHAVDIALMALDMLAGVGTFELQHLPGIPLWIRIGVHSGPCAAGVVGNK
Xenopus	AYMVVSGLPNRNGNNHAVDISRMALDILCFMGSFELRHLPGLPVWIRIGIHSGPCAAGVVGIK

Sequences obtained using the polymerase chain reaction. The degenerate primers were used in the PCR to amplify amino acids 894-956, 890-952, and 854-916 in the catalytic domains of GC-A, GC-B, and GC-C, respectively.

increase the activity of a particulate guanylyl cyclase in the intestinal brush-border. Elevated cyclic GMP is correlated with increased intestinal secretion, which results in the diarrhea associated with enterobacterial infection (Field *et al.*, 1978). Reports in the literature suggested that toxin binding and guanylyl cyclase activities reside in separate molecules, but chromatographic separation of the two activities was not complete. To determine whether a guanylyl cyclase is the ST receptor, degenerate primers were designed based on conserved sequences in the catalytic domain of all cloned guanylyl cyclases. These primers were used in the polymerase chain reaction (PCR) to amplify guanylyl cyclase sequences in the cDNA from rat small intestine. A new member of the cyclase family (GC-C) was identified by this method, and its cDNA subsequently cloned (Schulz *et al.*, 1990). GC-C conferred both ST binding and guanylyl cyclase activity on transfected COS cells (Schulz *et al.*, 1990). Recently, a 15 residue peptide, guanylin, was purified from acid extracts of rat small intestine (Currie *et al.*, 1992). Guanylin is 47% identical with the active portion of ST, and elevated cyclic GMP levels in T84, an intestinal cell-line rich in GC-C (Currie *et al.*, 1992). While the effects of guanylin on cloned GC-C have not yet been assessed, this peptide may, in fact, be its endogenous ligand.

Guanylyl Cyclase-Linked Receptors in Other Organisms

Natriuretic peptides have been identified in such diverse organisms as amphibians, fish, and birds (Table I), suggesting that receptors for these ligands are also present throughout the animal kingdom. We have used a PCR approach to identify guanylyl cyclases in a variety of species representing several phyla. RNA was prepared from various tissues of frog, fish, sea urchin, and earthworm. RNA was reverse transcribed to cDNA which was used as a PCR template with degenerate primers similar to those used to identify GC-C. These primers amplify a 250 base pair region in the highly conserved cyclase catalytic domain. Natriuretic peptide receptor-like sequences (homology > 70%) were amplified from frog, fish, and sea urchin; sequences homologous with GC-C were amplified from frog and fish (Table II). Additional sequences with homology to the membrane guanylyl cyclases, but with no strong homology to any particular receptor type, were amplified from both sea urchin and fish. Additional members of the receptor family may yet remain to be identified in mammals. Several guanylyl cyclase sequences were also amplified from earthworm. Although none of these sequences was strongly homologous with a particular mammalian cyclase, they were clearly of the membrane, rather than of the soluble type. An ANP-like peptide has been identified in earthworm heart by immunological cross-reactivity with mammalian ANP (Vesely and Giordano, 1992). This suggests that a homolog of GC-A may exist in invertebrates outside the Deuterostomia. Crustacean hyperglycemic hormone (CHH) seems to activate membrane guanylyl cyclase (Goy, 1990). CHH is a large peptide without apparent sequence homology to the natriuretic peptides or guanylin, although it does contain multiple cysteine residues. Thus, the Protostomia may have evolved an independent set of guanylyl cyclase receptors and ligands.

Summary

Guanylyl cyclase is a receptor for diverse intracellular and extracellular signalling molecules. A growing body of evidence suggests that multiple forms of this receptor-enzyme exist throughout the animal kingdom. Some receptor types are apparently conserved across species, oth-

ers may be unique. Studying the interaction between the guanylyl cyclases and their respective ligands will be valuable in defining the role of cyclic GMP in cellular function.

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